RESEARCH

Soybean Aphid Resistance in PI 243540 Is Controlled by a Single Dominant Gene

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ABSTRACT

The soybean aphid (*Aphis glycines* Matsumura) is a pest of soybean [*Glycine max* (L.) Merr.] in many soybean growing countries of the world. Host plant resistance is a very useful component of an integrated pest management program to control an insect problem. A maturity group (MG) IV plant introduction (PI) 243540 showed strong antibiosis resistance against the Ohio biotype of the soybean aphid. The objective of this study was to determine the inheritance of soybean aphid resistance gene(s) in PI 243540. The F_1, F_2, and F_2-derived F_3 families from a cross between an aphid susceptible cultivar Wyandot and resistant PI 243540 were screened in a greenhouse with the Ohio biotype of the soybean aphid. All F_1 plants were resistant to the soybean aphid and χ^2 analysis of segregation of 341 F_2 plants indicated a fit to a single dominant gene ratio of 3:1 ($P = 0.51$). Segregation in 330 $F_{2:3}$ families fit an expected 1:2:1 ratio ($P = 0.40$). Our results indicate that a single dominant gene controls the soybean aphid resistance in PI 243540. The simple inheritance of this gene should be helpful to quickly transfer the gene to susceptible elite cultivars using the backcross breeding approach.

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Abbreviations: IPM, integrated pest management; MG, maturity group; OARDC, Ohio Agricultural Research and Development Center; PCR, polymerase chain reaction; PI, plant introduction; SSR, simple sequence repeat.

The soybean aphid (*Aphis glycines* Matsumura) has been a pest of soybean [*Glycine max* (L.) Merr.] in North America since 2000 (Hartman et al., 2001). In 2003, significant yield losses of soybean in several midwestern states were attributed to the soybean aphid (Hill et al., 2006b). Nearly 80% of the soybean fields in the United States were infested by the soybean aphid by 2004 (Venette and Ragsdale, 2004). By 2005, soybean aphids were found in 23 soybean growing states of the United States and in many soybean fields in the North Central region soybean aphid numbers crossed the economic threshold. In 2003, nearly $150 million was spent for insecticides to control soybean aphids in infested soybean fields in the United States (Li et al., 2007). Severe soybean aphid infestation can reduce soybean seed yield by more than 50% (Ostlie, 2002; Wang et al., 1994). In addition to reduction of seed yield, soybean aphid can also reduce seed quality (e.g., discoloration, deformation) which is a major problem for food-grade soybean growers and in production of organic soybean (S. St. Martin, The Ohio State University, personal communication,

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2007). Another major concern is the ability of the soybean aphid to transmit certain plant viruses such as *Alfalfa mosaic virus*, *Soybean dwarf virus*, and *Soybean mosaic virus* to soybean (Iwaki et al., 1980; Hartman et al., 2001; Hill et al., 2001). The soybean aphid is the first soybean-colonizing aphid in the United States, and the full extent of its ability to transmit virus diseases among soybean plants as well as to and from other crop plants is still unknown.

Host plant resistance is a very useful component of an integrated pest management (IPM) program to control insect pests (Auclair, 1989). The soybean aphid is a new pest of soybean in the United States and at the time of its introduction no soybean aphid resistant cultivar was available in the United States. Thus, the soybean growers in the United States rely mainly on chemicals to control the soybean aphid. Chemical control of soybean aphids requires frequent scouting by trained individuals and use of established thresholds. Also, applying insecticides to soybean fields to control aphids can kill beneficial insects and may cause environmental pollution (Sun et al., 2000). Use of chemicals for controlling soybean aphids is also unacceptable to the producers and consumers of organic soybean products.

The first step in breeding a resistant cultivar is to identify a good source of resistance. Recently, four research groups have reported identification of soybean germplasm with resistance to the soybean aphid (Hill et al., 2004; Mensah et al., 2005; Diaz-Montano et al., 2006; Mian et al., 2008). Determination of the number of genes controlling the soybean aphid resistance in a new source of resistance and the mode of resistance (e.g., dominant, recessive, additive) is important for establishing breeding strategies. For example, qualitative traits require different breeding methods than quantitatively inherited traits. Hill et al. (2006a) reported that the soybean aphid resistance in cultivar Dowling was controlled by a single dominant gene named *Rag*1. Similarly, the soybean aphid resistance in cultivar Jackson was controlled by an unnamed single dominant gene (Hill et al., 2006b).

Recently, two biotypes of the soybean aphid—one from Illinois and one from Ohio—have been confirmed (Kim et al., 2008). While the soybean aphid resistance genes in Dowling and Jackson are still effective against the soybean aphid biotype from Illinois, these genes provides no protection against the Ohio biotype (Kim et al., 2008; Mian et al., 2008). Strong antibiosis resistance (i.e., feeding on the plant results in mortality or disruption of growth and development of the insect) to the Ohio biotype of soybean aphid was reported in a MG IV soybean accession PI 243540 (Mian et al., 2008). This PI was also resistant against the Illinois biotype of the soybean aphid (Mian et al., 2008). The PI 243540 is a potentially useful source for developing soybean cultivars with resistance to soybean aphids, particularly with resistance to the Ohio biotype of the soybean aphid. Knowledge of inheritance of the soybean aphid resistance gene(s) in PI 243540 will be useful for utilizing this soybean accession in developing aphid-resistant cultivars. The objective of this study was to determine the inheritance of the soybean aphid resistance gene(s) in PI 243540.

MATERIALS AND METHODS

Development and Screening of F_1 Lines

Crosses were made between the aphid-susceptible soybean cultivar Wyandot from Ohio (Mian et al., 2008) and aphid-resistant PI 243540 by transferring viable pollens from PI 243540 to the stigma of emasculated flowers of Wyandot. The F_1 progeny of the cross were screened for soybean aphid resistance in a greenhouse at the Ohio Agricultural Research and Development Center (OARDC) in Wooster, OH, during the fall of 2006 using the Ohio biotype of the soybean aphid. The source of the soybean aphid and the greenhouse conditions were described by Mian et al. (2008). Soybean aphids were collected from a soybean field near OARDC during the summer of 2005. The aphids were maintained and multiplied in growth chambers as described by Mian et al. (2008). A total of 13 F_1 seeds were produced from which 11 seedlings were obtained and two seeds did not germinate. Each F_1 plant was grown in a 25-cm-deep, 10-cm-diam. plastic pot filled with sterilized Pro-Mix soil (Premier Horticulture Ltd., Dorval, QC, Canada). Pots were arranged on a bench top with 15-cm spacing between rows and 12-cm spacing between plants within the rows. Three pots each with four to five seedlings of Wyandot as well as three pots each with four to five seedlings of PI 243540 were randomly placed among the F_1 plants. At the V1-stage (Fehr and Caviness, 1977), each seedling was infested with apterous aphids by placing an aphid-infested leaf or stem section with 20 to 30 soybean aphids between the petiole of the expanding trifoliate leaf and the stem of each seedling. Twenty-one days after infestation, each plant was assigned a visual score for soybean aphid colonization and plant health using the scoring system described by Hill et al. (2006b) with slight modifications. Each plant was assigned a score between 1 and 5, where 1 = no soybean aphid present; 2 = few (<25) solitary live or dead soybean aphid bodies present; 3 = some soybean aphids (between 25 and 100) with some viviparous aptera surrounded by few nymphs present; 4 = dense colonies on the upper half of the stem, underside of most leaves, and near the growing point of the plant with more than 300 soybean aphids; and 5 = similar or more severe soybean aphid infestation as in score 4 accompanied by visible plant damage (e.g., curled and/or yellow leaves, stunted growth).

After scoring, each plant was transplanted in a 7.6-L size plastic pot filled with the soil media and the plant was sprayed with an insecticide to kill the soybean aphids. The DNA from each F_1 plant was sampled and genotyped with two simple sequence repeat (SSR) markers determined to be polymorphic between the two parents for confirming the hybrid status of each plant. Polymerase chain reaction (PCR) amplifications were modified from the protocol of Diwan and Cregan (1997). Polymerase chain reaction consisted of initial denaturation at

94°C for 3 min, followed by 32 cycles of 45 s denaturation at 94°C, 45 s annealing at 47°C, and 45 s extension at 72°C followed by a 8 min final extension at 72°C on a thermocycler. The PCR products along with a 50 base pair size-standard were resolved by horizontal gel electrophoresis using 4% superfine resolution agarose (Ameresco, Solon, OH). The gels were stained with ethidium bromide and the gel images were captured with Genesnap (V.6.08) using the Gene Genius Bioimaging System (SYNGENE, Cambridge, UK). The SSR bands were scored manually from the saved gel images. The F_1 plants were grown to maturity and nearly 400 F_2 seeds were harvested from 11 F_1 plants.

Screening of F_2 Lines

During the early Spring of 2007, 346 F_2 seedlings were grown in the greenhouse in 3.8-L size pots, infested with the Ohio biotype of the soybean aphid, and assigned a score for soybean aphid resistance following the same procedures as described above. The greenhouse conditions and the source of soybean aphids were the same as described by Mian et al. (2008). A total of 10 pots (each with three to four seedlings) of each of the two parents were placed among the F_2 plants at regular intervals as checks. After scoring was completed, each F_2 plant was treated with an insecticide to kill the soybean aphids and was grown to maturity as a seed source. Soybean aphid susceptible plants were given special care with nutrient and water to make sure they recovered from the soybean aphid damage and were able to set seeds. Plants were kept under long day light hours (>15 h) until a full recovery and lush vegetative growth occurred. The plants then were placed under a 13-h day light period to initiate flowering. Plants were grown to maturity and the $F_{2:3}$ seeds from each F_2 plant were harvested in an envelope. Of the 346 F_2 plants, 11 produced insufficient seeds (i.e., less than 15 seeds) and five more plants had inconsistent results and these lines were not included in the final analysis of data.

Screening of $F_{2:3}$ Families

For each F_2 plant, a minimum of 12 F_3 seedlings were evaluated for segregation of soybean aphid resistance in a greenhouse screening using the Ohio biotype of the soybean aphid during the fall of 2007. The number of seedlings screened from each $F_{2:3}$ family ranged between 12 and 16 with an average of 14 seedlings per family. The greenhouse screening protocols were the same as described for the F_1 and F_2 plants screenings, except that six to eight seedlings were grown in each 3.8-L size plastic pots, thus two pots were used for each $F_{2:3}$ family. The pots were arranged at random on greenhouse benches and 10 pots (each with six to eight seedlings) of each parent were also placed randomly among the progeny pots. Three weeks after infestation each plant was scored for soybean aphid resistance using the 1 to 5 scale described earlier. Chi-square analysis was performed to test the goodness of fit of observed segregations among F_2 plants and $F_{2:3}$ families with different genetic ratios.

RESULTS AND DISCUSSION

F_1

The soybean aphid scores for all PI 243540 seedlings were ≤2. The Wyandot seedlings had ≥4 soybean aphid scores, except one seedling with a score of 3. A total of 11 F_1 plants were screened for soybean aphid resistance and all had the phenotype of the resistant parent PI 243540 (soybean aphid scores ≤2). The hybrid status of each F_1 plant was tested by genotyping with two SSR markers previously determined as polymorphic between the two parents. All F_1 plants were heterozygous with SSR bands from both parents (Fig. 1). The hybrid status of these progeny lines were also confirmed by the purple color of their flowers. The flower color of Wyandot dot is white and PI 243540 is purple.

F_2

Segregation of soybean aphid resistance among F_2 progeny fit the 3:1 resistant/susceptible genetic ratio ($P = 0.51$) (Table 1) indicating that a single dominant gene controlled soybean aphid resistance in PI 243540. Each of the 11 $F_{1:2}$ families of the cross segregated in a 3:1 resistant/susceptible ratio ($P = 0.12$–1.00) (Table 1). The soybean aphid scores for all PI 243540 plants were 1 or 2 while the soybean aphid scores for Wyandot plants ranged from 3 to 5. The progeny with scores of 1 or 2 were considered resistant while progeny with scores of 3 to 5 were considered susceptible, because PI 243540 plants always had scores of ≤2.0. The F_2 plants had soybean aphid scores of 1 to 5. Only six F_2 plants had the score of 3 and these six plants were confirmed as susceptible with all susceptible progeny in the corresponding $F_{2:3}$ families. Five F_2 plants scored as resistant were found susceptible in evaluation of the corresponding $F_{2:3}$ families. These five F_2 progeny probably escaped soybean aphid infestation despite our efforts to eliminate such escapes from the

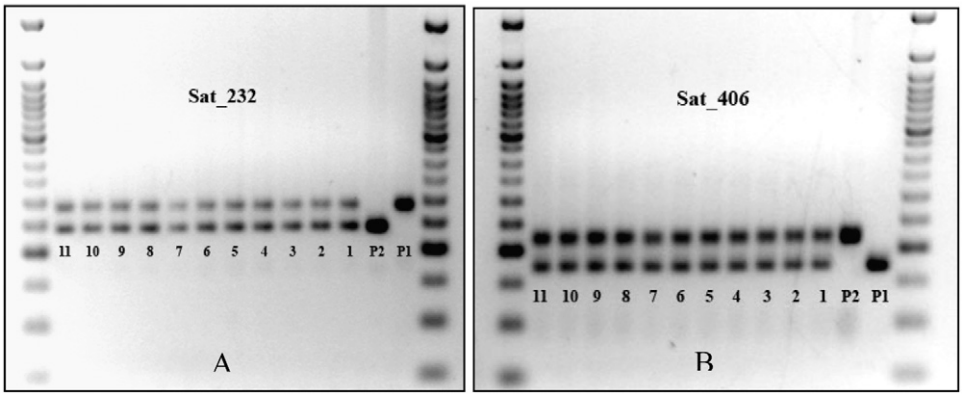

Figure 1. The agarose gel images (A) and (B) show simple sequence repeat bands from both parents to be present in the F_1 plants. P1, susceptible parent, Wyandot; P2, resistant parent, PI 243540; numbers 1 to 11, F_1 plants. The left-most and right-most lanes show a 50-bp size standard.

study. These F_2 plants and their progeny were dropped from further screenings and final statistical analysis was done using data from 341 F_2 plants.

$F_{2:3}$

Segregation analysis for soybean aphid resistance in 330 $F_{2:3}$ families of the Wyandot × PI 243540 cross showed a good fit to the expected 1:2:1 (93 homozygous resistant/160 heterozygous resistant/77 susceptible) ratio ($P = 0.40$) (Table 2) confirming that a single dominant gene controlled soybean aphid resistance in this population. All 11 F_1 plants produced families with similar segregation patterns (Table 2).

Recently Hill et al. (2006a) reported that the soybean aphid resistance in cultivar Dowling was controlled by a single dominant gene named *Rag*1. Similarly, the soybean aphid resistance in cultivar Jackson was controlled by an unnamed single dominant gene (Hill et al., 2006b). The results of this inheritance study clearly indicate that a single dominant gene controls the soybean aphid resistance in PI 243540. While PI 243540 is resistant to both Ohio and Illinois biotypes of the aphid, *Rag*1 from Dowling and unnamed resistance gene from Jackson provide resistance to the Illinois biotype only. These differential responses of the Ohio biotype of soybean aphid to these genes indicate that the soybean aphid resistance gene in PI 243540 is not *Rag*1 or the unnamed gene from Jackson. Li et al. (2007) have mapped *Rag*1 and the unnamed gene from Jackson to the same genomic region on soybean LG M indicating that these two resistance genes may be allelic. The status of the resistance gene in PI 243540 as a separate independent gene or a new allele of the soybean aphid resistance genes in Dowling or Jackson can be distinguished by molecularly mapping the gene in PI 243540 on the consensus soybean genetic map.

The simple inheritance of the soybean aphid resistance gene in PI 243540 should allow rapid introgression of this gene into aphid-susceptible but high-yielding U.S. soybean cultivars. It might be particularly useful to transfer the gene into cultivars known to be popular with organic soybean growers. We have already developed BC_4 lines by backcrossing this gene to a high-yielding Ohio soybean cultivar, Wyandot. The near qualitative and dominant nature of soybean aphid resistance of this gene made the selection of resistant progeny simple and easy. As this gene provides resistance against both the Illinois and Ohio biotypes of the soybean aphid it should be an attractive new resource of aphid resistance for soybean breeders and researchers in the United States and Canada.

Table 1. Segregation of 341 F_2 plants in 11 $F_{1:2}$ families for resistance to the Ohio biotype of soybean aphid in a population of Wyandot × PI 243540 cross.

$F_{1:2}$ family	No. of plants	Observed†		Expected (3:1)		χ^2 value	*P*
		Res.	Sus.	Res.	Sus.		
1	37	24	13	27.8	9.3	2.03	0.15
2	40	29	11	30.0	10.0	0.13	0.72
3	19	15	4	14.3	4.8	0.16	0.69
4	36	28	8	27.0	9.0	0.15	0.70
5	36	27	9	27.0	9.0	0.00	1.00
6	28	23	5	21.0	7.0	0.76	0.38
7	24	20	4	18.0	6.0	0.89	0.35
8	37	27	10	27.8	9.3	0.08	0.78
9	36	31	5	27.0	9.0	2.37	0.12
10	13	10	3	9.8	3.3	0.03	0.87
11	35	27	8	26.3	8.8	0.09	0.77
Pooled	341	261	80	255.8	85.3	0.43	0.51

†Resistant (Res.) soybean aphid scores 1 and 2 and susceptible (Sus.) soybean aphid scores 3, 4, and 5 where 1 = no soybean aphid present; 2 = few (<25) solitary live or dead soybean aphid bodies present; 3 = some soybean aphids (25–100) with some viviparous aptera surrounded by few nymphs present; 4 = dense colonies on the upper half of the stem, underside of most leaves, and near the growing point of the plant with more than 300 soybean aphids; and 5 = similar or more severe soybean aphid infestation as in score 4 accompanied by visible plant damage.

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Table 2. Segregation of 330 $F_{2:3}$ families originating from 11 $F_{1:2}$ families for resistance to the Ohio biotype of soybean aphid in a population of Wyandot × PI 243540 cross.

$F_{1:2}$ family	No. of $F_{2:3}$ families	Observed†			Expected (1:2:1)			χ^2	*P*
		Sus.	Het.	Res.	Sus.	Het.	Res.		
1	37	13	15	9	9.3	18.5	9.3	2.19	0.33
2	40	11	17	12	10.0	20.0	10.0	0.95	0.62
3	18	4	9	5	4.5	9.0	4.5	0.11	0.95
4	36	8	14	14	9.0	18.0	9.0	3.78	0.15
5	36	9	18	9	9.0	18.0	9.0	0.00	1.00
6	26	5	16	5	6.5	13.0	6.5	1.38	0.50
7	24	4	10	10	6.0	12.0	6.0	3.67	0.16
8	35	9	17	9	8.8	17.5	8.8	0.03	0.99
9	34	5	17	12	8.5	17.0	8.5	2.88	0.24
10	11	2	6	3	2.8	5.5	2.8	0.27	0.87
11	33	7	21	5	8.3	16.5	8.3	2.70	0.26
Pooled	330	77	160	93	82.5	165.0	82.5	1.85	0.40

†Resistant (Res.) soybean aphid scores 1 and 2 and susceptible (Sus.) soybean aphid scores 3, 4, and 5 where 1 = no soybean aphid present; 2 = few (<25) solitary live or dead soybean aphid bodies present; 3 = some soybean aphids (25–100) with some viviparous aptera surrounded by few nymphs present; 4 = dense colonies on the upper half of the stem, underside of most leaves, and near the growing point of the plant with more than 300 soybean aphids; and 5 = similar or more severe soybean aphid infestation as in score 4 accompanied by visible plant damage. Het., heterozygous.

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